

## University of Groningen

### Kathepsine C

Gorter, Jeannette

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

1969

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Gorter, J. (1969). *Kathepsine C: Een allosterisch enzyme*. s.n.

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

## SUMMARY

In Chapter I an introduction into allosteric systems is given. In Chapter II a detailed method is described for the application of Gly-Phe-*p*.nitroanilide (GPNA) as a substrate for the activity assay of the lysosomal enzyme cathepsin C. It is an allosteric enzyme which is activated by  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CNS}^-$ ,  $\text{NO}_3^-$ , and  $\text{ClO}_3^-$  ions. (Chapter III). At high activator concentrations the enzyme obeys Michaelis-Menten kinetics, at low concentrations there is substrate cooperativity. Thus, in the absence of added activators a small amount of the substrate Ala-Phe-amide enhances the hydrolysis rate of a low concentration of GPNA; at higher concentrations it is a competitive inhibitor.

At very low substrate concentrations, where cooperativity does not play a role, the activity depends on the activator concentration according to the simple scheme  $\text{E} + \text{A} \xrightleftharpoons{K_a} \text{E}_a$ . The activators differ in their affinity constants  $K_a$  as well as in their maximum activity values, the latter in the order  $\text{Cl}^- > \text{Br}^- > \text{I}^- = \text{CNS}^- = \text{NO}_3^- = \text{ClO}_3^-$ . The residual activity without halide activators is very low and might be due to traces of chloride. Very high concentrations of halides (0.5 M) except chloride are inhibitory.

Halide activation very likely affects the substrate affinity, not the catalytic rate constants. Imidazole in its protonated form behaves as an inhibiting substrate analogue; the inhibition by imidazole decreases with decreasing chloride concentration, thus supporting this view.

In the presence of chloride ions cathepsin C is much more stable towards heat denaturation than in its absence.

Cathepsin C is a potent catalyst for transamidation reactions in which amino acceptors are coupled to dipeptide acyl-donors (Chapter IV). We found the enzyme to have a definite specificity for the amino acceptor. The specificity rules found with amino acceptors have much in common with the rules found with the parts of tri- and tetrapeptide substrates at the amino part of the susceptible amide bond. In transamidation reactions the acceptor peptide probably occupies the binding site for the amino part of the substrate in hydrolysis. It is highly unlikely that transamidation occurs *in vivo*.

With GPNA the rate constant of acylation  $k_2$  and the rate constant of deacylation  $k_3$  are of the same order of magnitude, in contrast with amide substrates such as Gly-Phe-amide, in which deacylation is rate-limiting (Chapter V).  $k_3$  has a constant value throughout the pH range (3.7 - 7.6) investigated. The  $k_2$  value of GPNA decreases above pH 5.  $K_s$  is dependent on a group with pK 4.0 which must be in its dissociated form. It is assumed that this group, very likely a carboxylate group, is involved in binding of the N-terminal protonated group of the substrate. The rather sizable active site of the enzyme is discussed in Chapter VI.

It is still obscure whether cathepsin C has a general or a more specific function in intracellular protein or peptide degradation. We are convinced that chloride controls cathepsin C activity *in vivo*. However, the chloride concentration in lysosomes is unknown. A biological role of the cooperativity is, in our opinion, improbable.

In Chapter VI some other known halide-activated enzymes are compared to cathepsin C.